Week 9

August 1, 2016

Gibson Plate Counts

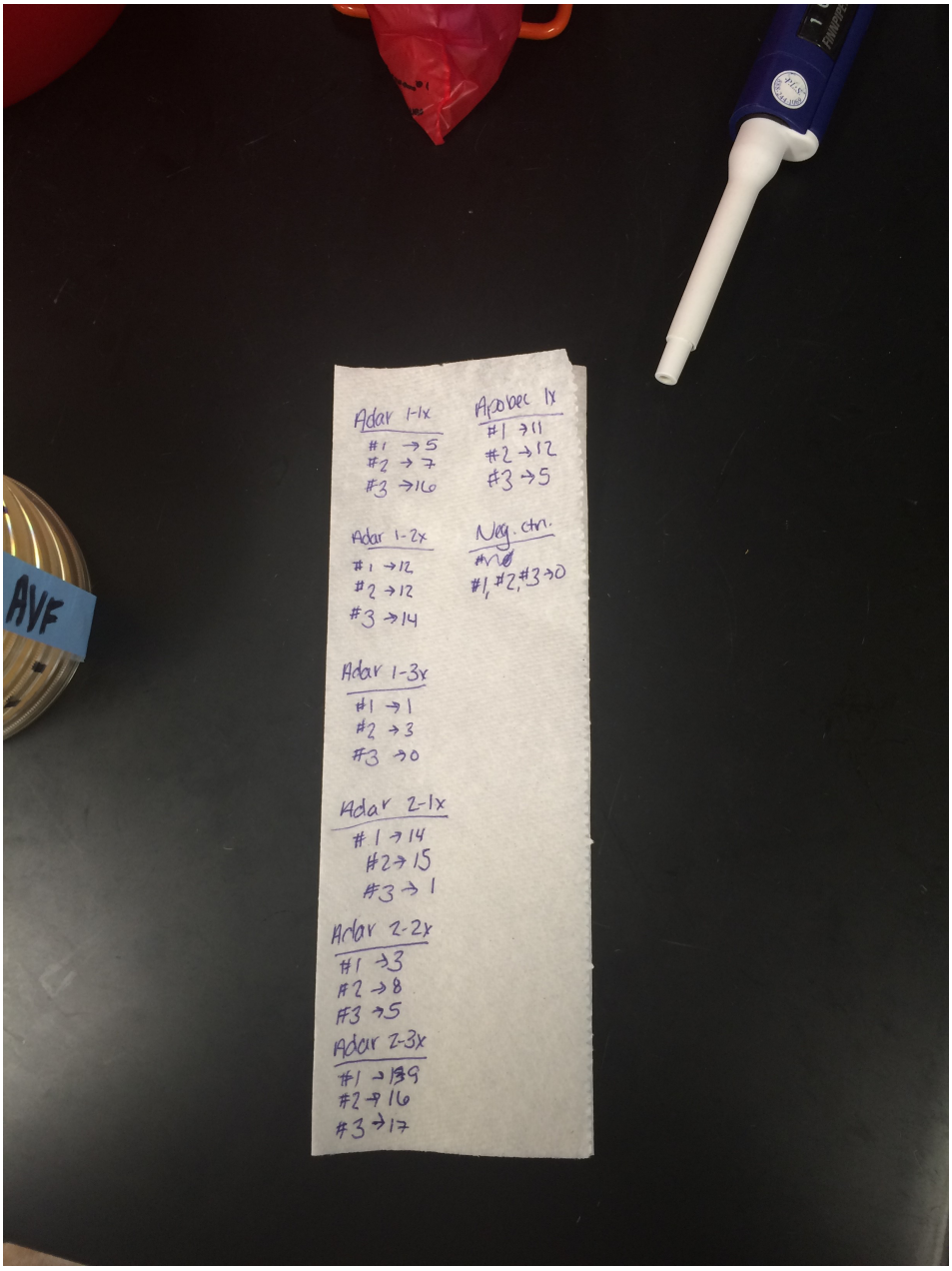


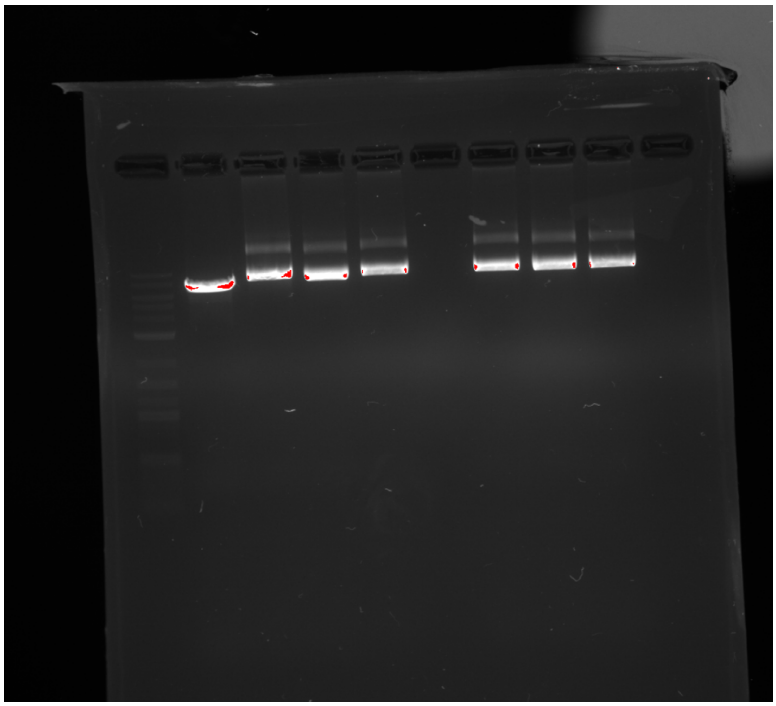
Plate cells today for transfection Tuesday with Apobec 2x and 3x

Wiki day
For the test digests of last week

The only two enzymes that gave a small amount of background were BamHI and HindIII
HEK293T cell maintenance
Created 2 12 well plates for fluorescence test and western blot

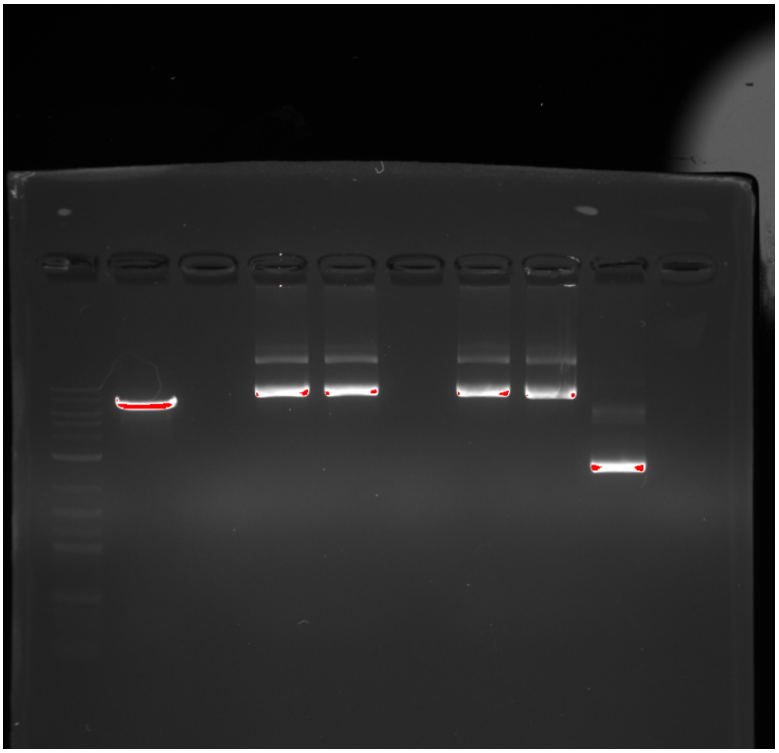
- Data analysis
    - eGFP trial 1 slides
    - ImageJ
    - Excel

August 2, 2016



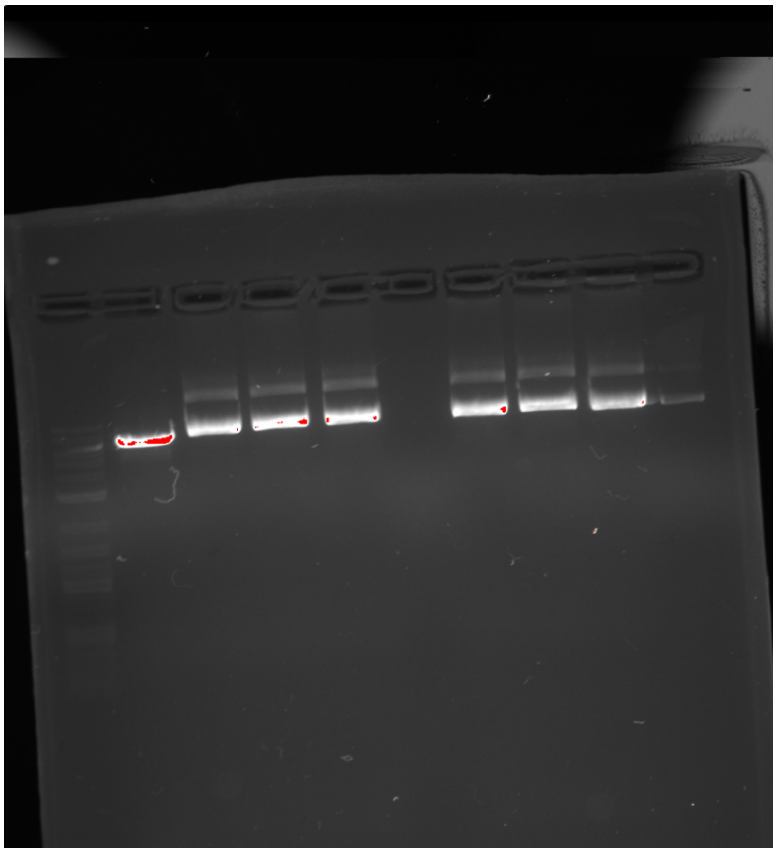
Gel 1
Lane 1 – Ladder
Lane 2 – Adar 1-1x #1
Lane 3 – Adar 1-1x #2
Lane 4 – Adar 1-1x #3
Lane 5 – Empty
Lane 6 – Adar 1-2x #1
Lane 7 – Adar 1-2x #2
Lane 8 – Adar 1-2x #3

Analysis
They ran too small.



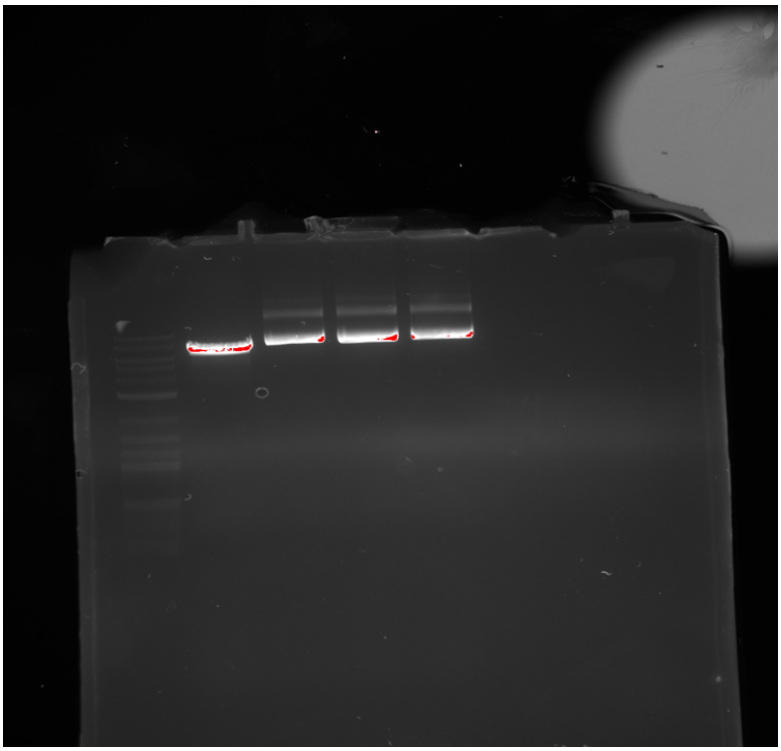
Gel 2
Lane 1 – Ladder
Lane 2 – Empty
Lane 3 – Adar 1-3x #1
Lane 4 – Adar 1-3x #2
Lane 5 – Empty
Lane 6 – Adar 2-1x #1
Lane 7 – Adar 2-1x #2
Lane 8 – Adar 2-1x #3

Analysis
The gels ran too small.
Especially something is wrong in Lane 8.



Gel 3
Lane 1 – Ladder
Lane 2 – Adar 2-2x #1
Lane 3 – Adar 2-2x #2
Lane 4 – Adar 2-2x #3
Lane 5 – Empty
Lane 6 – Adar 2-3x #1
Lane 7 – Adar 2-3x #2
Lane 8 – Adar 2-3x #3

Analysis
Lanes ran too small.



Gel 4
Lane 1 – Ladder
Lane 2 – Apobec 1x #1
Lane 2 – Apobec 1x #2
Lane 3 - Apobec 1x #3

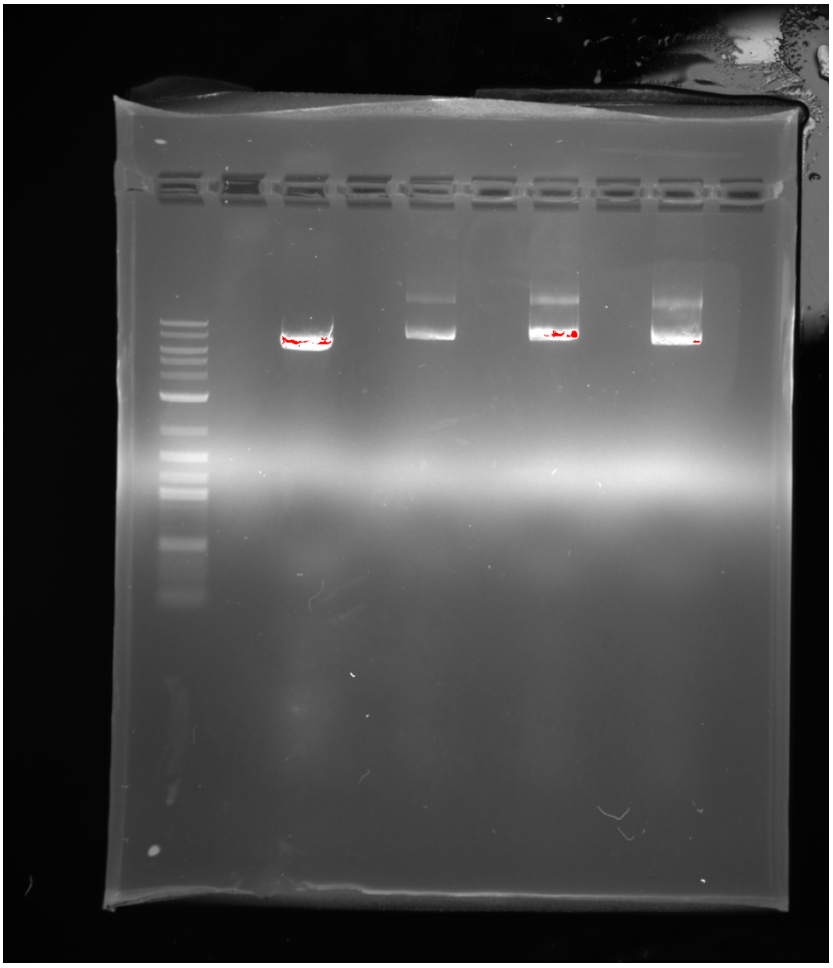
Analysis
Lanes ran too small.

- New cell culture media
  100 mL
- Cell plating
  Cell count: 327; 395 → Average = 361
  - 1.444 * 10^6 cells/mL

  Plating:
  - 12-well plate: 3*10^5 cells * 13 wells → 2.7 mL
  - 4 flasks: 3*10^5 cells * 13 wells → 4.16 mL
- Transfection:
  APOBEC plate
  - Row 1: pRetro empty
  - Row 2: pRetro-APO2X-dCas9
  - Row 3: pRetro-APO3X-dCas9

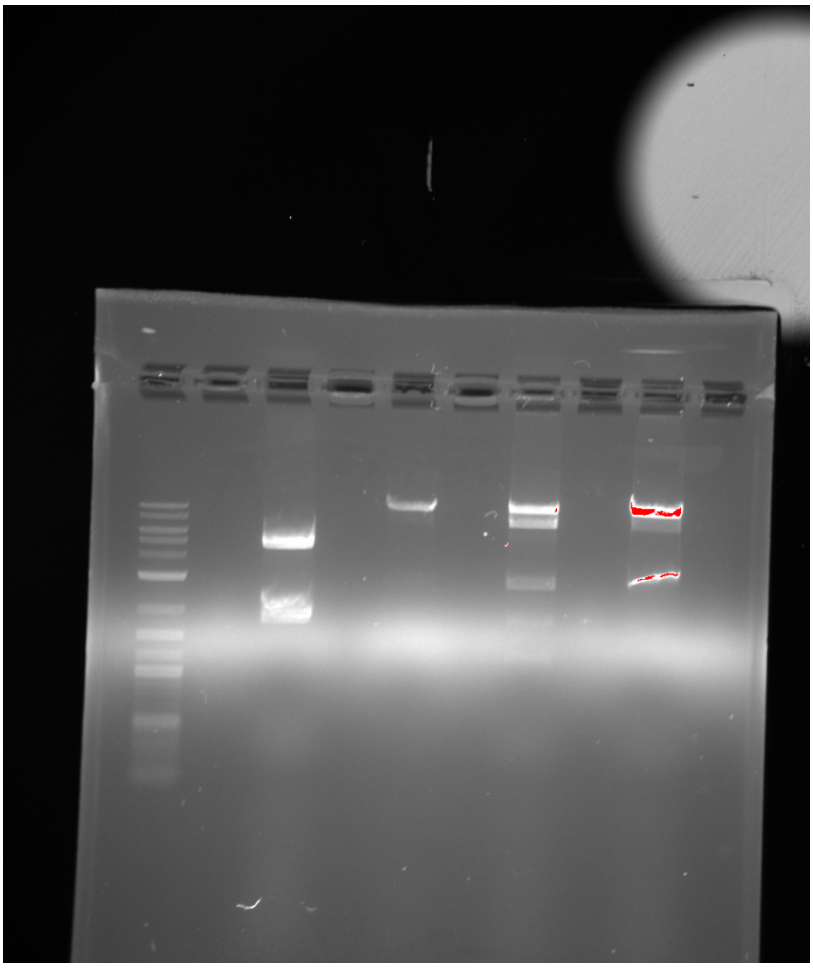
Transfected HEK293T cells with pcDNA, peGFPN3, ATG+ACG eGFP
Created cultures for interlab in CAM

August 3, 2016



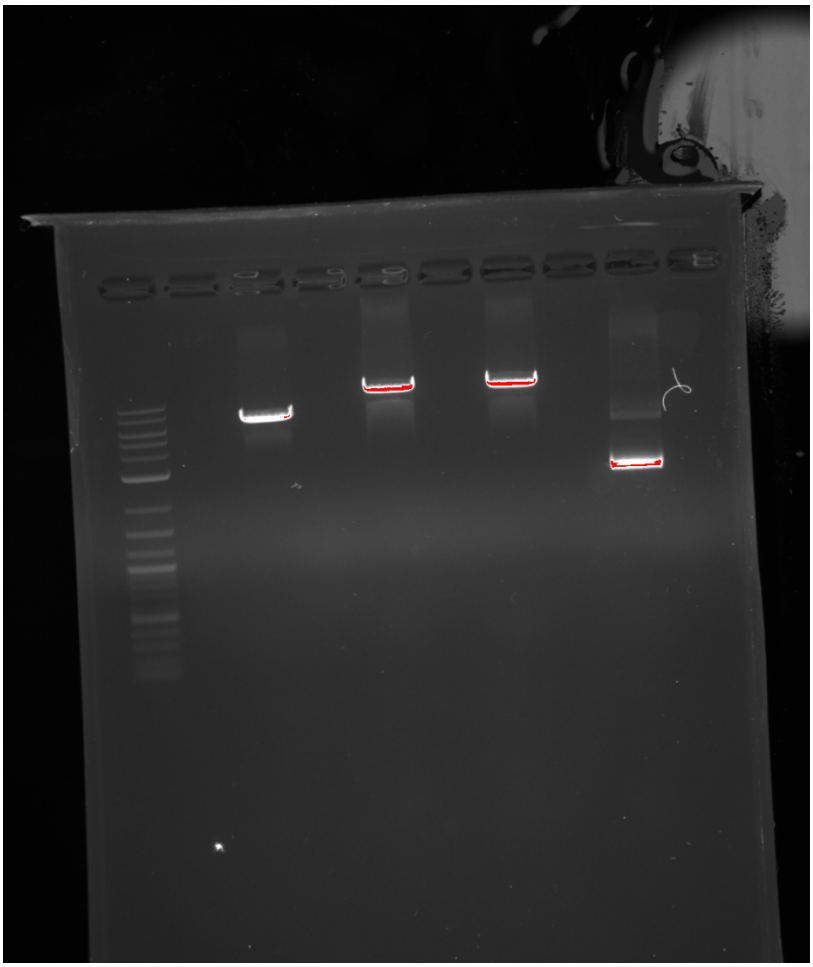
Gel 1 – Digest with only xho1
Lane 1 – Ladder
Lane 2 – Empty
Lane 3 – Neg. Control
Lane 4 – Empty
Lane 5 – Positive Control (using Apobec clone)
Lane 6 – Empty
Lane 7 – Adar 1-3x #1
Lane 8 – Empty
Lane 9 – Adar 2-1x #2

Analysis
They all ran too small, even the positive control, which is known to be a clone. Something may be wrong with the xho1 cut cite or the enzyme itself.



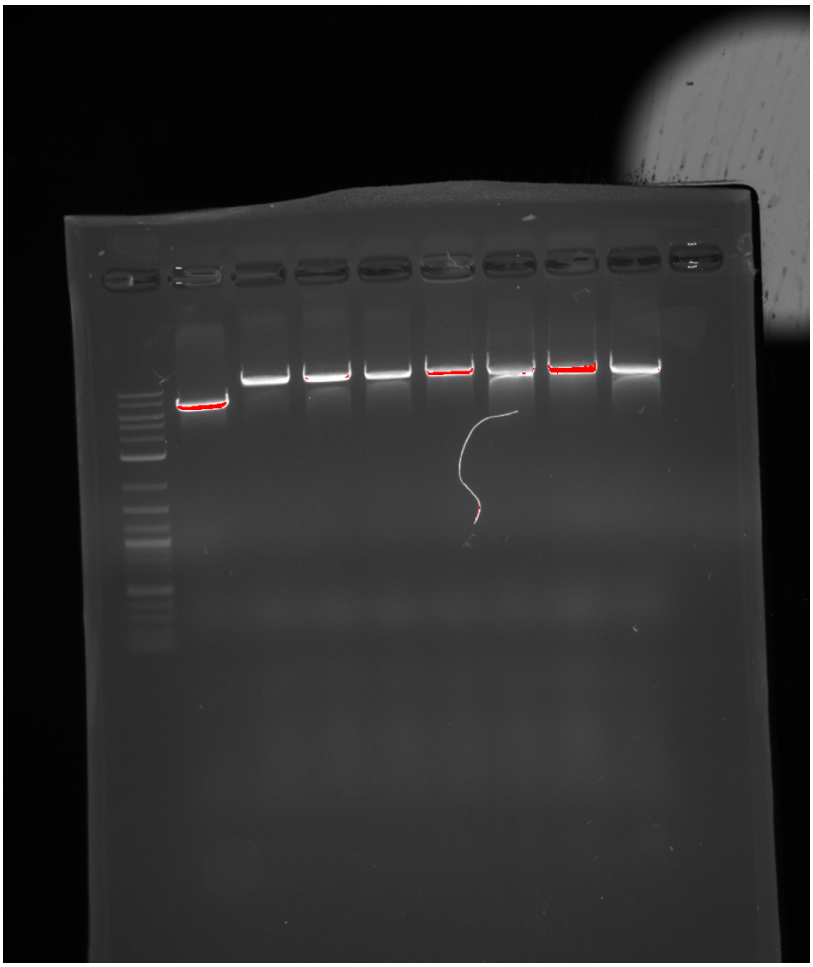
Gel 2 – Digest with xho1 and EcoR1
Lane 1 – Ladder
Lane 2 – Empty
Lane 3 – Neg. Control
Lane 4 – Empty
Lane 5 – Positive Control
Lane 6 – Empty
Lane 7 – Adar 1-3x #1
Lane 8 – Empty
Lane 9 – Adar 2-1x #2

Analysis
The clones ran correctly for having the dCas9 insert and the editing enzyme insert. We will run a gel with just EcoR1 to see if something is wrong with xho1.



Gel 3 – Digest with only EcoR1
Lane 1 – Ladder
Lane 2 – Empty
Lane 3 – Negative Control
Lane 4 – Empty
Lane 5 – Positive Control
Lane 6 – Adar 1-2x #1
Lane 7 – Empty
Lane 8 – Adar 2-1x #3

Analysis
The positive control and Adar 1-2x #1 ran correctly (they have dCas9 and the editing enzyme in the vector). However Adar 201x #3 did not run correctly. We can assume from this that there is something wrong with the xho1 enzyme. We will do a test digest with only EcoR1 with the rest of the clones to test them.



Gel 5 – Digest of all Clones with EcoR1 only
Lane 1 – Ladder
Lane 2 – Neg. Control
Lane 3 - Adar 1-1x #1
Lane 4 – Adar 1-2x #1
Lane 5 – Adar 1-3x #2
Lane 6 – Adar 2-1x #2
Lane 7 – Adar 2-2x #1
Lane 8 – Adar 2-3x #3
Lane 9 – Apobec 1x #3

- Transfection
  - eGFP trial 3
  - 1 µg pgRNA + 3 µg of:
    - pEGFP N3 (+)
    - pcDNA (–)
    - ATG GFP (ATG)
    - ACG GFP (ACG)
  - Adjust to 200 µL with 0.1X TE → add 200 µL 2X HBS
  - Add 100 µL to corresponding well
- Fixing cells
  - eGFP trial 2
- New cell culture media
  - 200 mL
- Induction
  - Doxycycline → APOBEC plate
    - Column 1: 0
    - Column 2: 10 ng/mL
    - Column 3: 100 ng/mL
    - Column 4: 1000 ng/mL

Transfection, fixed cells
Performed Interlab Experiment

August 4, 2016

- Imaging
  - eGFP trial 2
- Cell plating
  - Cell count: 335; 323 → Average = 329
    - $1.316 * 10^6$ cells/mL
  - Plating:
    - 12-well plate: $3*10^5$ cells * 13 wells → 2.96 mL cells
- Harvest APOBEC plate (for Western blot)
  - 1.5 mL 2xSDS sample buffer + 30 µL DTT
  - Heat at 80 ˚C, 10 min
  - Store in freezer
- Gibson of globin prep
- PCR amplified globin
- Interlab Data Management
- PCR'd ACG/ATG eGFP using biobrick primers

August 5, 2016

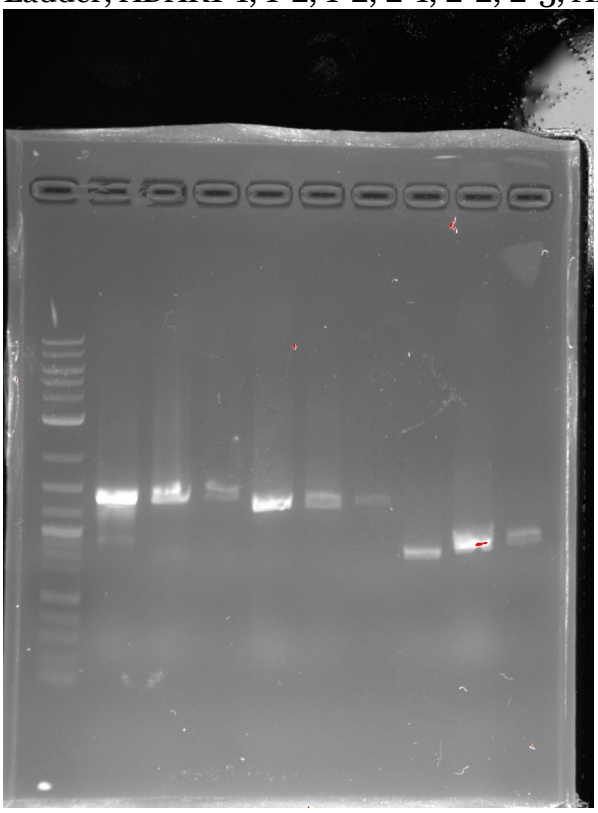
Ran globin on gel
PCR purified globin
PCR enzymes for biobrick

Gibson of globin onto Kan plates (3uL vector, 6uL insert)
Gibson on gRNA to AMP plates
(1uL vector, 9uL insert)

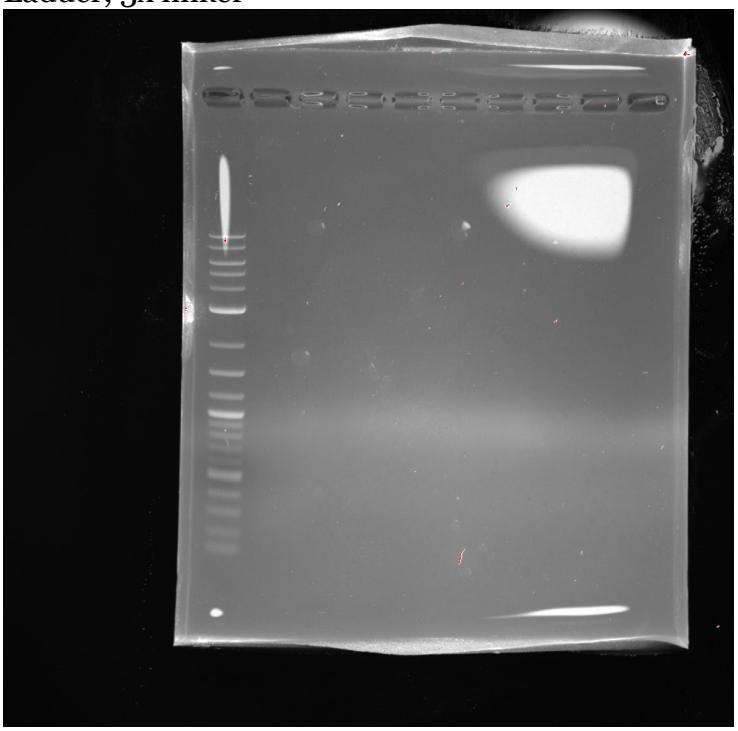
Key:
purple ACG sgRNA
Red globin sgRNA
brown scramble
black and purple sgRna neg control
green WT
orange PTC
black globin neg control

PCR order
ADAR1-1, 1-2, 1-2, 2-1, 2-2, 2-3, APOBEC1, 2, 3, 3x linker

Gel 1:
Ladder, ADAR1-1, 1-2, 1-2, 2-1, 2-2, 2-3, APOBEC1, 2, 3, 3x



Gel 2:
Ladder, 3x linker



Nothing there

Tested PCR products for eGFP biobrick parts
Gibson PCR product into BioBrick plasmids

- Fixing cells
  - eGFP trial 3
- Transfection
  - eGFP trial 2 (redo)
- Imaging
  - Exposure: Green = 26.17; Red = 1432
  - 46 images total